Short Communication

Semiquantitative Human Papillomavirus Type 16 Viral Load and the Prospective Risk of Cervical Precancer and Cancer

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Abstract

We examined whether higher human papillomavirus type 16 (HPV16) viral load predicted risk of cervical intraepithelial neoplasia 3 (CIN3) or cancer (together termed ≥CIN3) within a cohort of 20,810 women followed for 10 years with cytologic screening. Semiquantitative viral load for HPV16 was measured on baseline cervicovaginal specimens using a type-specific hybridization probe test with signal amplification. An increased risk of ≥CIN3 associated with higher HPV16 viral load was found only among cytologically negative women in early follow-up, suggesting that these cases were related to the detection of prevalent lesions missed at baseline. Women with higher HPV16 viral load were more likely to undergo ablative treatment during follow-up than those with lower viral load (P_{trend} = 0.008), possibly diminishing any additional risk for ≥CIN3 attributable to higher HPV16 viral loads. (Cancer Epidemiol Biomarkers Prev 2005;14(5): 1311–4)

Introduction

Cervical infection by a group of ~15 oncogenic human papillomavirus (HPV) types causes virtually all cervical cancers worldwide (1-3). HPV is a common sexually transmitted agent (4, 5) that typically clears within 1 to 2 years. Many HPV infections do not cause detectable cytologic abnormalities (6, 7); however, some infections persist and progress over several years to high-grade cervical intraepithelial neoplasia (CIN3). Left untreated, a substantial proportion of these precancers eventually develop into invasive cervical cancer (8).

High HPV viral load has been shown to be associated with the microscopic diagnosis of a concurrent lesion (9-14). In addition, high HPV viral load, especially HPV16 viral load, may be an indicator that an HPV infection is likely to progress to precancer and cancer. In a case-control study, Josefsson et al. (15) reported that women with increasingly higher HPV16 viral load, measured using quantitative PCR on cells scraped from archival Pap smears, were at an increasingly greater risk for development of carcinoma *in situ* (roughly equivalent to CIN3) up to 13 years before diagnosis. In a related study, repeat detection of high HPV16 viral load by quantitative PCR has been associated with increased risk of carcinoma *in situ* compared with women with low HPV16 viral load (16). Another study using quantitative PCR found that high HPV16 viral load predicted progression to CIN2/CIN3 and conferred a decreased likelihood of viral clearance compared with low viral load (17). Finally, a study using a PCR-based method for a semiquantitative assessment of viral load in cervical scrapes found that among women with no evidence of cytologic squamous intraepithelial lesions (SIL), high viral load was associated with a 3- to 5-fold increase risk for the development of cytologic high-grade SIL during a follow-up period of up to 8 years compared with low viral load (18).

In contrast, we reported no link between semiquantitative baseline measurement of oncogenic HPV viral load for a pool of oncogenic types and subsequent histologic CIN3 or cancer (≥CIN3) in a 10-year cohort study of women attending a health maintenance organization in Portland, Oregon (19). In that study, we used a hybridization and signal amplification–based probe cocktail to test for 13 oncogenic HPV types (Hybrid Capture 2, Digene Corporation, Gaithersburg, MD); values progressively greater than the positive cut point were considered as a surrogate for oncogenic HPV viral load *in toto*, which has been shown to correlate with quantitative PCR–measured viral load (20, 21). In an attempt to synthesize the apparently discrepant results from our group and others, we recently retested the Hybrid Capture 2 test positives from our earlier study specifically for HPV16 using a semiquantitative test method (22). Specifically, we wished to determine whether increased viral load of HPV16 specifically might convey an appreciably greater risk of ≥CIN3.

Materials and Methods

Study Subjects. Between April 1, 1989, and November 2, 1990, 23,702 women were enrolled in a natural history study of HPV infection at the Kaiser Permanente, Northwest Region prepaid health plan in Portland, Oregon, as previously described (23, 24). Both NIH and Kaiser Permanente Institutional Review Boards approved the study. Subjects were ages ≥16 years with a mean age of 35.9 years (range 16-94 years). A main analysis cohort of 20,810 women was established and followed passively as part of standard cytologic screening for cervical neoplasia (24). This analysis cohort excluded women

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Table 1. Relationships between HPV viral load and development of ≥CIN3 during a 10-year follow-up

Time after baseline (mo)	Viral load (RLU/PC)	Women seen	Women with CIN3+	Absolute risk	RR (95% CI) {All}	RR (95% CI) {cytologic negative}	RR (95% CI) {ASCUS or LSIL}
0-<9	0.60-1.33	241	4	1.7	1.0 (reference group)	1.0 (reference group)	1.0 (reference group)
	1.34-4.32	162	9	5.6	3.4 (1.1-11)	0	1.3 (0.40-4.1)
	4.33-21.11	161	9	5.6	3.4 (1.1-11)	5.1 (0.53-48)	1.2 (0.34-4.1)
	≥21.12	135	12	8.9	5.4 (1.8-16)	8.2 (0.87-78)	0.98 (0.30-3.2)
9-<69	0.60-1.33	198	7	3.5	1.0 (reference group)	1.0 (reference group)	1.0 (reference group)
	1.34-4.32	120	8	6.7	1.9 (0.71-5.0)	3.3 (1.1-9.7)	0
	4.33-21.11	120	7	5.8	1.7 (0.57-4.8)	2.1 (0.63-7.1)	0.46 (0.05-4.5)
	≥21.12	93	6	6.5	1.8 (0.63-5.3)	3.1 (0.93-10)	0.19 (0.02-1.9)
69-122	0.60-1.33	129	1	0.8	1.0 (reference group)	1.0 (reference group)	N/A
	1.34-4.32	73	1	1.4	1.8 (0.11-28)	2.5 (0.16-38)	N/A
	4.33-21.11	72	1	1.4	1.8 (0.11-28)	1.9 (0.12-29)	N/A
	≥21.12	40	0	0.0	0	0	N/A
9-122	0.60-1.33	197	8	4.1	1.0 (reference group)	1.0 (reference group)	1.0 (reference group)
	1.34-4.32	124	9	7.3	1.8 (0.71-4.5)	2.9 (1.1-8.0)	0
	4.33-21.11	123	7	5.7	1.4 (0.52-3.7)	1.7 (0.57-5.2)	0.46 (0.05-4.5)
	≥21.12	95	7	7.4	1.8 (0.65-5.1)	3.0 (0.96-9.5)	0.18 (0.02-1.8)
Overall	0.60-1.33	242	12	5.0	1.0 (reference group)	1.0 (reference group)	1.0 (reference group)
	1.34-4.32	163	18	11.0	2.2 (1.1-4.5)	2.4 (0.94-6.4)	0.83 (0.33-2.1)
	4.33-21.11	165	17	10.3	2.1 (1.0-4.3)	2.1 (0.82-5.6)	0.84 (0.31-2.3)
	≥21.12	134	18	13.4	2.7 (1.4-5.5)	3.6 (1.3-9.6)	0.69 (0.27-1.8)

Abbreviation: N/A, not applicable because no cases observed in the reference group.

who (*a*) refused to participate (n = 1,107), (*b*) had undergone hysterectomy (n = 1,406), (*c*) had an inadequate specimen for HPV testing (n = 195), (*d*) had unsatisfactory or missing enrollment cervical smears (n = 85), or (*e*) underwent colposcopy rather than Pap smear screening at enrollment (n = 99).

Enrollment Examination. According to Kaiser Institutional Review Board guidelines, subjects underwent a routine pelvic examination (23). Exfoliated cervical cells were typically collected with an Ayre spatula and a cytobrush for Pap test screening. Next, each subject underwent a cervicovaginal lavage to collect specimens for HPV testing. Lavages were done by rinsing the cervical os with 10 mL sterile physiologic saline using a syringe fitted with an intracatheter extender and then collecting the pooled fluid in the vaginal fornix using the same device (23). Lavages were refrigerated within 1 hour of collection and shipped to a central laboratory for processing (22, 23).

HPV16 Testing. We tested frozen aliquots (−70°C) of lavages from a stratified sample[5] of 4,321 women for HPV16 DNA by a type-specific adaptation of the hybrid capture method using HPV16-specific capture oligonucleotides and a HPV16 RNA probe as previously reported (22). Signal strengths in relative light units (RLU) were compared with 1 pg/mL HPV16 DNA–positive controls (RLU/PC). Based on a receiver operating characteristic analysis, a 0.6 RLU/PC (pg/mL) positive cut point was selected (22). The progression of numerical RLU/PC values above the positive cut point was taken to represent a surrogate for HPV viral load as shown in previous studies (20).

Follow-up. Subjects were followed up to 122 months, undergoing a median of three repeat smears, with 83% of women having at least one repeat smear. Women with negative enrollment cervical smears had a median follow-up of 6 years. Those with abnormal cytology were managed according to standard practice guidelines; HPV data were not unmasked or used for clinical management.

As detailed in the results, this report focuses on a group of 516 (2.5%) of the 20,810 baseline cohort members who had satisfactory Pap smears and tested positive for HPV16 DNA using a single type-specific probe for HPV16 (see below). Of these 516 HPV16-positive women, 64 (12.4%) were diagnosed with ≥CIN3 histology during follow-up using a rigorous pathologic definition involving independent review (25).

Analysis. Follow-up time was crudely divided into an initial period of 9 months (Pap smears that were rapidly repeated, presumably prompted by a previous cytologic abnormality or suspicious symptoms) followed by yearly intervals for a total time of 122 months. These 12-month intervals roughly paralleled the intervals at which women returned for annual smears. As a consequence of a small number of newly diagnosed cases during follow-up, we then combined time intervals into periods of 0 to <9, 9 to <69, and 69 to 122 months, which would categorize outcomes as prevalent, early-incident, and late-incident disease.

We stratified semiquantitative viral loads among HPV16-positive women into quartiles (0.60-1.33, 1.34-4.33, 4.33-21.11, and ≥21.12 RLU/PC) to examine the association of viral load and risk of ≥CIN3. We also used strata defined by log units of viral load (0.60-5.99, 6.00-59.99, 60.00-599.99, and ≥600 RLU/PC). Extrapolating from testing results for the sampling fractions to the entire population (an estimated 699 HPV16 positives; ref. 11), we calculated absolute risk and relative risk (RR) for ≥CIN3 with 95% confidence intervals (95% CI) for three time periods (0-<9, 9-<69, and 69-122 months), for 9 to 122 months, and for the entire 122-month follow-up. Confidence intervals were based on the actual number of women tested. Results were also stratified on baseline cytologic interpretations [cytologic negative (including reactive changes) or atypical squamous cells of unknown significance (ASCUS)/low-grade SIL (LSIL)].

To evaluate whether there was any bias in the frequency of screening or any censoring related to viral load, we examined the relationships of HPV16 viral load to number of screening visits, to having a mild cytologic abnormality, and to undergoing ablative surgery during follow-up for the 452 HPV16-positive women who did not become cases during follow-up (<CIN3). Pearson χ^2 test and the Mantel extension test for trend were used to evaluate the relationships of viral load and these follow-up screening and treatment characteristics.

[5]All 171 cases of ≥CIN3 diagnosed over the 10-year study; 867 women who were not diagnosed with ≥CIN3 but who were previously reported to be HPV DNA positive by MY09/11 L1 consensus primer PCR (n = 855 results, 98.6%); all 2,260 women who were either not tested or negative by PCR but positive by Hybrid Capture 2 (n = 2,253 results, 99.7%); 23 women with an enrollment Pap smear interpreted as LSIL or more severe and not captured by the above strata (n = 23 results, 100%); a 6% random sample of the remaining 17,489 women not included in the above strata was tested (n = 1043, 100%).

Results

For the entire 122-month follow-up, women with the highest three quartiles of HPV16 viral load had similarly elevated RRs for ≥CIN3 of 2.2 (95% CI, 1.1-4.5; 2nd quartile), 2.1 (95% CI, 1.0-4.3; 3rd quartile), and 2.7 (95%,CI, 1.4-5.5; 4th quartile) compared with the lowest quartile of viral load (Table 1). The greatest period of elevation of risk for ≥CIN3 was the first 9 months with RRs for ≥CIN3 of 3.4 (95% CI, 1.1-11; 2nd quartile), 3.4 (95% CI, 1.1-11; 3rd quartile), and 5.4 (95% CI, 1.8-16; 4th quartile). Stratified on cytologic interpretation at baseline, cytologic-negative women with higher HPV16 viral loads were at elevated risk of ≥CIN3 during intervals of 0 to <9 and 9 to <69 months, but there was again no difference in risk between the upper three quartiles of viral load. No elevated risk for higher viral loads was observed for women with ASCUS/LSIL cytology at baseline. Using strata defined by log units of viral load tended to mute the risk of ≥CIN3 associated with high viral load (data not shown).

Table 2. Relationships of HPV16 viral load to the number of screening visits (A), to any mild cytologic abnormality (B), and to undergoing an ablative treatment (C) during follow-up of noncases

A. Number of screening visits during follow-up

Viral load (RLU/PC)	1-2 Visits	3-4 Visits	5-7 Visits	8 Visits	Total
0.60-1.33	39	39	25	26	117
	33.3%	23.1%	21.4%	22.2%	
1.34-4.32	42	26	23	19	110
	38.2%	23.6%	20.9%	17.3%	
4.33-21.11	50	24	17	23	114
	43.9%	21.1%	14.9%	20.2%	
21.12	35	25	31	20	111
	31.5%	22.5%	27.9%	18.0%	
Total	166	102	96	88	452

$P = 0.5$, Pearson χ^2; $P_{trend} = 0.9$

B. Any mild cytologic abnormality during follow-up

Viral load (RLU/PC)	No	Yes	Total
0.60-1.33	95	22	117
	81%	19%	
1.34-4.32	89	21	110
	81%	19%	
4.33-21.11	89	25	114
	78%	22%	
21.12	68	43	111
	61%	39%	
Total	303	149	452

$P = 0.001$, Pearson χ^2; $P_{trend} = 0.001$

C. Underwent surgery during follow-up

Viral load (RLU/PC)	No	Yes	Total
0.60-1.33	89	28	117
	76%	24%	
1.34-4.32	71	39	110
	65%	35%	
4.33-21.11	80	34	114
	70%	30%	
21.12	63	48	111
	57%	43%	
Total	303	149	452

$P = 0.02$, Pearson χ^2; $P_{trend} = 0.008$

NOTE: The bottom set of numbers for each viral load range are row percentages.

Among the noncases, we examined the effects of HPV16 viral load on follow-up (Table 2). We found no relationship between viral load and the number of follow-up screening visits ($P_{trend} = 0.9$), suggesting that there was no significant difference in women's screening behavior according to their viral load. However, women with higher HPV16 viral loads were both more likely to have a cytologic abnormality ($P_{trend} = 0.001$) and have ablative surgery ($P_{trend} = 0.008$) during follow-up. Inclusion of cases did not alter these findings (data not shown).

Discussion

We found that HPV16-infected women with higher RLU/PC signal strength over a broad range were at a slightly higher risk of ≥CIN3 than women with the lowest signal strength. This finding differs from our previous finding (19) in which we did not observe an elevated risk for women with higher viral load for a pooled probe test (Hybrid Capture 2) for 13 oncogenic types that included HPV16. Although this finding is consistent with the idea that higher viral load for HPV16 confers elevated risk for ≥CIN3, we observed the greatest risk elevation within the first 9 months of follow-up, suggesting that most of these cases were missed prevalent at baseline but detected with a delay at follow-up. This is consistent with earlier findings in which high viral load, especially for HPV16, was elevated among those of concurrent abnormal pathology (9-14, 26); the relationship of degree of severity and viral load is less certain (11) perhaps because of the influence of low-grade lesions on the viral load measurement for high-grade lesions (27).

The elevated risk was primarily observed in women who were cytologically negative or had benign reactive changes. In our large cohort, there was no added risk attributed to higher HPV16 viral load for women with equivocal (ASCUS) or mildly abnormal (LSIL) cytology. Given the well-recognized relationship of abnormal cytology and histologically diagnosed CIN with higher HPV viral loads (11), it seems likely that women with abnormal cytology overall had higher viral loads as we found to be true in this study (Kruskal-Wallis, $P = 0.0001$). Thus, cytologic abnormality and higher viral load are ''correlates'' of each other, and knowing either one reduces the predictive value (for ≥CIN3) of knowing the other.

We note that observed increases in risk were quite modest, with no stepwise trend, compared with those previously reported (15, 17, 18). We offer several explanations. First, by using a signal amplification–based HPV test as a surrogate for viral load, we did not detect the extreme low end of viral load that can be detected by quantitative PCR. The cut point for these single probes and pooled probe assays were selected based on a receiver operating characteristic analysis versus high-grade cervical neoplasia and cancer and, thus, represent the optimal clinical cut point for detection of prevalent high-grade cervical neoplasia rather than mere detection of HPV DNA. Therefore, infections detected by quantitative PCR with a very high analytic sensitivity that would have been called negative by hybridization methods likely represent extremely low viral load, possibly clinically irrelevant infections. Thus, using extremely low viral load infections (an equivalent of using a lower cut point) as the reference group would have inflated estimates of risk for all HPV viral load levels detected.

We also found significant correlation between increased viral load and ablative treatments. Women in the Portland Kaiser plan commonly underwent ablative treatments for cytologic abnormalities in accordance with an aggressive clinical management. Among controls, women who had higher viral loads, whether for HPV16 or another type (data not shown), were more likely to have abnormal cytology follow-up that resulted in ablative treatment. If ablative treatment tends to censor women at highest risk, as suggested by the data and

clinical judgment, estimates of absolute risk and RR of progression to ≥CIN3 reflect local screening and clinical practice (19); particularly, we expect the effects of viral load on the risk of ≥CIN3 in an aggressive and effective cytology screening program, like Portland Kaiser's, to be muted compared with the effect in a setting with a less effective program. The relatively poor sensitivity of cytology, coupled with long screening intervals in some countries with less aggressive screening and clinical management than the United States, may produce a stronger association of HPV viral load with missed occult CIN3 that is later diagnosed and interpreted as incident disease. To further examine these issues, we are now evaluating the impact of HPV16 and HPV18 viral load in our population study in Guanacaste, Costa Rica, where management of cytologic abnormalities is less conservative.[6]

Finally, some prior studies may have considered some LSIL and atypical squamous cell cytology as negative. It is now apparent that there are significant regional/national differences in threshold of cytologic abnormality (28). HPV-positive cytology is more likely to be reclassified as SIL upon review than HPV-negative cytology, with an attendant higher risk of subsequent CIN3 and cancer.[7]

Despite the large size of our 20,000-woman HPV-screened cohort, one of the largest research cohorts reported, only 516 HPV16 infections were identified at enrollment in the sampling fraction and a small number of cases. Consequently, we had unstable estimates of risk with wide confidence intervals.

Despite this limitation, we have shown that the association between high HPV16 viral load and risk of ≥CIN3 was too weak to form the basis for clinical management in this population. Differences in the definition of HPV16 positivity (and, therefore, the definition of the reference viral load category) and in the threshold (sensitivity) for cytologic abnormalities make the meaning of viral load too uninformative, given the tendency to treat women before the advent of CIN3 in a well-screened population.

[6] Gravitt et al., in preparation.

[7] Thus, the increased risk for high-grade cervical neoplasia and cervical cancer attributed to higher HPV16 viral load in other studies may be partly ascribed to comparing women who may have abnormal cytology but were called cytologic negative using more conservative standards of cytology to those who were cytologic negative by most standards.

References

1. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International Biological Study on Cervical Cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87:796–802.
2. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518–27.
3. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–9.
4. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218–26.
5. Koutsky L. Epidemiology of genital human papillomavirus infection. Am J Med 1997;102:3–8.
6. Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 2001;285:2995–3002.
7. Castle PE, Wacholder S, Sherman ME, et al. Absolute risk of a subsequent abnormal pap among oncogenic human papillomavirus DNA-positive, cytologically negative women. Cancer 2002;95:2145–51.
8. McIndoe WA, McLean MR, Jones RW, Mullins PR. The invasive potential of carcinoma *in situ* of the cervix. Obstet Gynecol 1984;64:451–8.
9. Cuzick J, Terry G, Ho L, Hollingworth T, Anderson M. Type-specific human papillomavirus DNA in abnormal smears as a predictor of high-grade cervical intraepithelial neoplasia. Br J Cancer 1994;69:167–71.
10. Cuzick J, Terry G, Ho L, Hollingworth T, Anderson M. Human papillomavirus type 16 in cervical smears as predictor of high-grade cervical intraepithelial neoplasia [corrected]. Lancet 1992;339:959–60.
11. Schiffman M, Herrero R, Hildesheim A, et al. HPV DNA testing in cervical cancer screening: results from women in a high-risk province of Costa Rica. JAMA 2000;283:87–93.
12. Wang SS, Hildesheim A. Chapter 5: Viral and host factors in human papillomavirus persistence and progression. J Natl Cancer Inst Monogr 2003;31:35–40.
13. Snijders PJ, van den Brule AJ, Meijer CJ. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J Pathol 2003;201:1–6.
14. Iftner T, Villa LL. Chapter 12: Human papillomavirus technologies. J Natl Cancer Inst Monogr 2003;31:80–8.
15. Josefsson AM, Magnusson PK, Ylitalo N, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma *in situ*: a nested case-control study. Lancet 2000;355:2189–93.
16. Ylitalo N, Sorensen P, Josefsson AM, et al. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma *in situ*: a nested case-control study. Lancet 2000;355:2194–8.
17. van Duin M, Snijders PJ, Schrijnemakers HF, et al. Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. Int J Cancer 2002;98:590–5.
18. Schlecht NF, Trevisan A, Duarte-Franco E, et al. Viral load as a predictor of the risk of cervical intraepithelial neoplasia. Int J Cancer 2003;103:519–24.
19. Lorincz AT, Castle PE, Sherman ME, et al. Viral load of human papillomavirus and risk of CIN3 or cervical cancer. Lancet 2002;360:228–9.
20. Gravitt PE, Burk RD, Lorincz A, et al. A comparison between real-time polymerase chain reaction and hybrid capture 2 for human papillomavirus DNA quantitation. Cancer Epidemiol Biomarkers Prev 2003;12:477–84.
21. Pretet JL, Dalstein V, Monnier-Benoit S, Delpeut S, Mougin C. High risk HPV load estimated by Hybrid Capture II((R)) correlates with HPV16 load measured by real-time PCR in cervical smears of HPV16-infected women. J Clin Virol 2004;31:140–7.
22. Castle PE, Lorincz AT, Scott DR, et al. Comparison between prototype hybrid capture 3 and hybrid capture 2 human papillomavirus DNA assays for detection of high-grade cervical intraepithelial neoplasia and cancer. J Clin Microbiol 2003;41:4022–30.
23. Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993;85:958–64.
24. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003;95:46–52.
25. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology, human papillomavirus testing, and risk for cervical neoplasia: a 10-year cohort analysis. J Natl Cancer Inst 2003;95:46–52.
26. Swan DC, Tucker RA, Tortolero-Luna G, et al. Human papillomavirus (HPV) DNA copy number is dependent on grade of cervical disease and HPV type. J Clin Microbiol 1999;37:1030–4.
27. Sherman ME, Wang SS, Wheeler CM, et al. Determinants of human papillomavirus load among women with histological cervical intraepithelial neoplasia 3: dominant impact of surrounding low-grade lesions. Cancer Epidemiol Biomarkers Prev 2003;12:1038–44.
28. Scott DR, Hagmar B, Maddox P, et al. Use of human papillomavirus DNA testing to compare equivocal cervical cytologic interpretations in the United States, Scandinavia, and the United Kingdom. Cancer 2002;96:14–20.